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Effects of $I_{k,Ach}$ Channel Inhibitor Tertiapin-Q on Right Atrial Preparations from Patients in Sinus Rhythm and Atrial Fibrillation

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Background: The acetylcholine-activated inward rectifying current, $I_{K,Ach}$, has been suggested as a novel atrial-selective putative drug target for the treatment of atrial fibrillation (AF). Here we have studied the effects of a selective $I_{K,ACh}$ channel inhibitor tertiapin-Q in human right atrial preparations from patients in sinus rhythm (SR) and permanent chronic atrial fibrillation (AF, >6 months).

Methods: Action potentials (APs) were measured using sharp electrodes in right atrial trabeculae or patch electrodes in cardiomyocytes from patients undergoing open heart surgery (48 in SR and 34 in AF).

Results and Conclusion: Tertiapin-Q (300nM) did not affect APs recorded in AF trabeculae (APD_{90} at 1Hz: control 205.8 ± 9.0ms *vs.* drug 209.0 ± 9.0ms, n.s.). Consistent with electrical remodelling of $I_{K,ACh}$ channels in AF, 1μM carbachol caused minimal effect on APD in AF trabeculae and no further effect was detected following combined exposure to carbachol and tertiapin-Q (APD_{90} 242.0 ± 16.9ms, 233.0 ± 20.5ms, 234.0 ± 19.1ms, respectively). In SR trabeculae, tertiapin-Q modestly, though not significantly, attenuated the shortening effect of carbachol on APD_{90}: 310.0 ± 7.7ms, 215.0 ± 16.4ms, and 254.0 ± 17.8ms, respectively). In the continuous presence of carbachol and tetrodotoxin (1μM), tertiapin-Q prolonged effective refractory period and APD_{90}. In atrial cardiomyocytes from SR patients, tertiapin-Q fully reversed the carbachol-induced shortening of APD_{90}. At a holding potential of −80mV, tertiapin-Q had no effect on the basal inward current in SR cardiomyocytes (2.0 ± 0.5pA/pF vs. 2.5 ± 0.7pA/pF, n=7), but significantly reduced in AF cardiomyocytes (6.8 ± 1.1pA/pF vs. 5.9 ± 0.8pA/pF, $P<0.05$, n=12), providing evidence for constitutive activity of $I_{K,ACh}$ in AF cardiomyocytes. From the modest effects of tertiapin-Q in multicellular preparations in comparison to cardiomyocytes, we conclude that access of the $I_{K,ACh}$ channel inhibitor to its target may be limited in intact superfused tissues.

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Modulation of Pancreatic Islet Electrophysiology and Insulin Release by Potassium Channel Subunit Kvbeta2

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Voltage-gated potassium channels (Kv) regulate pancreatic β-cell excitability, and thereby insulin secretion. Kv channels are modulated by ancillary cytoplasmic subunits, such as the Kvβ proteins. In this study, we investigated the electrophysiology of pancreatic β-cells and isolated islet function in mice lacking the Kvβ2 gene.

Methods: Islets from $Kv\beta2^{-/-}$ (KO) and wild-type (WT) mice were enzymatically isolated and disrupted into single cells. Whole-cell voltage clamp was used to measure Kv kinetics. Action potentials were recorded using the perforated-patch technique. Glucose stimulated insulin secretion was measured in isolated WT and KO islets. Mice underwent metabolic characterization.

Results: Kvβ2 expression in WT islets was demonstrated by qRT-PCR and Western. Compared with cells from WT mice, KO β-cells had a depolarized half-activation voltage ($V_{1/2\text{-act}}$), $p<0.01$. The Kv-blocker 4-aminopyridine (4AP) was used to characterize the Kv current. The IC50 of the 4AP-sensitive current was 190μM. Total β-cell Kv amplitude blocked by 4AP was 34.1 ± 2.6% at 500μM. Together, this suggests Kv1-family proteins may contribute significantly to β-cell repolarization. Kvβ2 is known to modulate Kv1-family currents by increasing surface expression, shifting $V_{1/2\text{-act}}$, and altering channel inactivation. Insulin release from KO islets at 2.5mM glucose, 3.26 ± 0.25 ng/mL/hr/5 islets, was greater than in WT islets, 0.64 ± 0.23 ng/mL/hr, $p=0.002$. Fasting plasma insulin was higher in KO than in WT, $p<0.025$, and KO had lower fasting blood glucose, $p<0.0001$. An intraperitoneal injection of glucose showed KO to have enhanced glucose tolerance as compared to WT. When housed in metabolic cages, KO mice had higher metabolic rates than age-matched WT.

Conclusion: Deletion of Kvβ2 alters the metabolic phenotype and may be related to increased release of insulin by the murine pancreas due to altered β-cell repolarization. These findings reveal a critical role of Kvβ2 in regulating insulin release.

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Pharmacological Consequences of PKC Inhibition on Kv1.5+Kvbeta1.3 Channels

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The $K_v\beta1.3$ subunit modifies the gating and pharmacology of $K_v1.5$ channels in a PKC-dependent manner, decreasing channel sensitivity to bupivacaine- and quinidine-mediated blockade. Cardiac $K_v1.5$ channels associate with receptor for activated C kinase 1 (RACK1), the $K_v\beta1.3$ subunit and different PKC isoforms, resulting in the formation of a functional channelosome. The aim of the present study was to investigate the effects of PKC inhibition on bupivacaine and quinidine block of $K_v1.5 + K_v\beta1.3$ channels. HEK293 cells were transfected with $K_v1.5 + K_v\beta1.3$ channels, and currents were recorded using the whole-cell configuration of the patch-clamp technique. PKC inhibition was achieved by incubating the cells with either calphostin C or bisindolylmaleimide II and the effects of bupivacaine and quinidine were analysed. The voltage-dependent inactivation of $K_v1.5 + K_v\beta1.3$ channels and their pharmacological behavior after PKC inhibition with calphostin C were similar to those displayed by $K_v1.5$ channels alone. Indeed, the IC_{50} values for bupivacaine were similar in cells whose PKC was inhibited with calphostin C or bisindolylmaleimide II. Similar results were also observed in the presence of quinidine. The finding that the voltage-dependence of inactivation and the pharmacology of $K_v1.5 + K_v\beta1.3$ channels after PKC inhibition resembled that observed in $K_v1.5$ channels suggests that both processes are dependent on PKC-mediated phosphorylation. These results may have clinical relevance in diseases that are characterized by alterations in kinase activity.
Supported by SAF2010-14916, SAF2013-45800-R and FIS-RIC RD12/0042/0019 Grants.

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Intersubunit Interactions Control Kir Channel Inactivation

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Inward rectifier potassium (Kir) channels are expressed in numerous mammalian tissues including the pancreas, brain, heart, and skeletal muscle and play a critical role in controlling cellular excitability. Pancreatic ATP-sensitive Kir (KATP) channels are key regulators of insulin secretion as they link cellular metabolism with membrane excitability. Loss-of-function (LOF) mutations in KATP can cause human hyperinsulinism as a result of diminished activity. Several of these LOF mutations disrupt salt-bridge interactions that are located within the intracellular subunit-subunit interfaces, and result in channel activity showing a fast inactivation following ATP removal as measured with the inside-out patch clamp technique. Inactivation can be subsequently abolished by application of PIP2 to the cytoplasmic face of the membrane, an action that can be explained by a simple model in which PIP2 competes with the closed inactivated state. We find that homologous mutations in the strong inward rectifying Kir2.1 channel cause lower basal activity as a result of reduced apparent PIP2 sensitivity, indicating increased inactivation. Kir2.1 channels contain additional intersubunit salt-bridge interactions that are not present in KATP channels. Introduction of these salt-bridges into the inactivating KATP channel mutants partially rescues the channel from this inactivating phenotype. These results lead us to propose that the stability of the intersubunit interface regulates channel inactivation, sensitivity to PIP2, and is conserved across the Kir channel family and stabilizing the intersubunit interface provides a potential strategy to exploit in development of activating modulators of KATP and other Kir channels

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Inhibition of Hsp70 Enhances A-Type Kv4 Current by Reducing Degradation of Auxiliary KChP4A

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We have previously shown that KChIP4s reduces surface expression of Kv4 through its N-terminal KID (Kv4 inhibitory domain) that causes endoplasmic reticulum retention of the channel complex. In this study we found that treatment of HEK 293 cells expressing KChIP4a with cycloheximide that inhibits protein synthesis causes a significant degradation of